Remarks

Claims 1-3, and 5-12 remain pending after entry of this amendment. Claims 1, 7, and 8 are withdraw from consideration. Claim 2 was amended herein. Claims 9-12 were added herein. Support for newly added claim 9 can be found at least in the claims as originally filed in combination with page 5, lines 12-17. Support for newly added claims 10, 11, and 12 can be found in the claims as originally filed, page 27 of the specification, and page 5, lines 12-17 of the specification. Favorable reconsideration is respectfully requested in light of the amendments and remarks submitted herein.

As a preliminary matter, Applicant notes that the Examiner may not consider newly added claims 10 - 12 as included in the same invention as that previously elected. Applicant is aware that the Office does not permit the Applicant, as a matter of right, to shift to claiming another invention via the filing of a RCE (MPEP § 819). However, Applicant would like to point out that the Office may waive the election and permit the shift. According to MPEP § 819.01, the Office is not precluded from permitting a shift, and it may do so where the shift results in no additional work or expense, and particularly where the shift reduces work as by simplifying the issues. Applicant respectfully requests the Examiner to consider newly added claims 10 - 12 along with the other elected claims because the shift will not result in any additional work or expense and will simplify the issues. Applicant respectfully asserts that the consideration of newly added claims 10 - 12 will simplify the issues because these claims are allowable in light of the cited art, and the art that has already been cited and considered is also the art that would be uncovered and relevant in a separate search carried out for these specific claims.

Claims 2-6 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Boyer et al. (U), Boyer et al. (V) in view of Horikoshi et al. (A), Colaruotolo et al. (B), Boyer et al. (IDS), Takowa et al. (IDS), and Jones (IDS). Applicant respectfully traverses this rejection.

Claim 2 has been amended to specify that the composition that is being prepared is useful for the treatment of alkaline textile industry waste water. Applicant respectfully asserts that this added recitation does provide a meaningful limitation, and also respectfully asserts that the cited references do not concern themselves with alkaline textile industry waste water. Therefore,

Applicant respectfully asserts that amended claim 2, and dependent claims 3-6 are not obvious in light of the above cited references.

Newly added claim 9, on the other hand includes the recitation of *Bacillus alkalophilus* CBTCC/Micro/8 and *Bacillus sp.* CBTCC/Micro/9. None of the cited references disclose or suggest these two strains because the Applicant discovered them. The strains have been deposited at MTCC, Chandigarh, India, which is recognized under the Budapest Treaty for the International Recognition of the Deposit of microorganisms. Specifically, *Bacillus alkalophilus* CBTCC/Micro/8 has the deposit number MTCC 5092, and a deposition date of March 17, 2003. *bacillus sp.* CBTCC/Micro/9 on the other hand has the deposit number MTCC 5093 and was also deposited on March 17, 2003. Therefore, Applicant respectfully asserts that this claim is also non-obvious.

Newly added claims 10-12 provide methods of treating alkaline textile industry waste waters. None of the cited references provide any disclosure or suggestion regarding textile industry waste waters. Therefore, Applicant respectfully asserts that these claims are also non-obvious in view of the cited references.

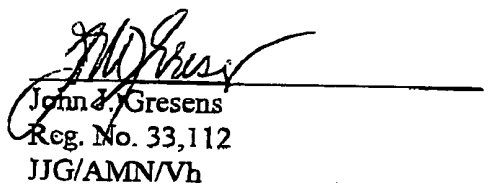
Conclusion

In view of the above amendments and remarks, Applicant respectfully requests a Notice of Allowance. If the Examiner believes a telephone conference would advance the prosecution of this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

Respectfully submitted,

MERCHANT& GOULD P.C.
P.O. Box 2903
Minneapolis, MN 55402-0903
612/332-5300

Date: 2 December 2003



John J. Gresens
Reg. No. 33,112
JJG/AMN/Vh

3/24

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Kumar et al. Serial No.: 09/867,367 Filed: 29th May 2001 For: *A Microbial composition and a process for the neutralization of alkaline waste waters*	Group Art Unit: 1617 Examiner: Shengjun Wang Attorney Docket No: 11378.14USC1

To,
The Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. § 1.132 by Rita Kumar

I, Rita Kumar, age 51 years, residing at C 6B/79,Janak Puri, New Delhi, INDIA, citizen of India do hereby state as under.

1. I am a Scientist at IGIB (previously CBT) , Mall Road, New Delhi, India. I was graduated in the year 1971 from J & K University located at Srinagar, INDIA. I completed my Master's Degree in 1973-74 from Haryana Agricultural University at HISSAR, INDIA in the year 1973-74. Subsequently, I was graduated with a doctoral degree in 1982 from Delhi University in the year 1982-1983.

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2. I took up my first assignment as a Scientist with CBT in year 1976. I am continuing to now work with CBT for about 26 years. I have been a Senior Scientist with CBT since 1988.

3. One of the projects undertaken by CBT is "A Microbial composition and a process for the neutralization of alkaline waste waters". This project was undertaken in the year 1992. The scientists involved in the study were Rita Kumar (myself), Anil Kumar and Alka Sharma. I was one of the main scientists in this study. I am aware of US patent application No. 09/867,367 filed in respect of this project. I am also aware and familiar with all the office actions, objections of the Examiner and the references cited by the Examiner. Therefore, I am completely and fully aware of all the facts relating to this project as well as the present patent application.

4. The relevant documents for the deposition of microbial strains and also for establishing the viability of strains are **attached herewith.** The strains were deposited at MTCC, Chandigarh, INDIA, recognized as per the Budapest Treaty on the International Recognition of the Deposit of microorganisms for the purpose of patent procedure. The details of the deposited strains are as follows:

Strain Bacteria	Previous deposition No.(CBTCC)	Deposition at MTCC No.	Date
1. ***Bacillus alkalophilus*** (having characteristics similar like to ATCC no. 27647)	CBTCC/Micro/8	MTCC 5092	17.03.2003
2. ***Bacillus sp*** (having characteristics similar like to ATCC no. 27557)	CBTCC/Micro/9	MTCC 5093	17.03.2003

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5. Regarding the question for the similarity between our strains and the two strains deposited at ATCC, I wish to submit that our strains have been isolated from Indian origin and were initially identified by biochemical tests only. The strains were found to be sort of having similar like characteristics to that of ATCC strains. However, they are not identical to the strains deposited at ATCC.

6. Further, both the strains are individually novel. Thus, the combining of novel strains further imparts novelty to the combination.

7. Regarding the question of synergistic effect, I would state that the synergistic effect is obvious from the data wherein additive effect is shown only when the two strains are used together. The results are quite significant in this regard. The additive effect is first step towards synergy. If it would not have been exhibiting synergistic effect which means that only one strain is predominating and in that case only the effect of one strain would be there, whereas in this case both the strains are working together and exhibiting their effect. It is also agreeable that the multiplicity of effect is not there, however it is established that multiplicity effect is not always expected in synergism . Absence of synergism could also lead to competitive inhibition between the two strains resulting in negative results, which are not the case in our studies.

8. The strains of the instant Application were tried to neutralize the alkaline wastewater from the beverages. However, the results were not encouraging. The results are as given below.

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Table 1 Bacterial Strain MTCC 5092 is unable to neutralize the beverage waste water when inoculated individually

Source	Original pH of Waste Water	After 2 days of incubation		After 4 days of incubation		After 7 days of incubation	
		pH	Change in pH unit	pH	Change in pH unit	pH	Change in pH unit
Without carbohydrates	12.43	12.41	0.02	12.40	0.03	12.38	0.05
	11.50	11.46	0.04	11.42	0..08	11.40	0.10
	10.91	10.83	0.05	10.80	0.11	10.78	0.13
	10.51	10.42	0.09	10.37	0.14	10.38	0.13
1% Sucrose	12.12	12.11	0.01	12.09	0.03	12.07	0.05
	11.50	11.47	0.03	11.40	0.10	11.38	0.12
	10.91	10.88	0.03	10.74	0.17	10.61	0.30
	10.51	10.47	0.04	10.31	0.20	10.20	0.31
1% Glucose	12.22	12.19	0.03	12.19	0.03	12.14	0.08
	11.50	11.46	0.04	11.40	0.10	11.40	0.10
	10.91	10.41	0.50	10.11	0.80	10.02	0.89
	10.51	10.12	0.39	9.97	0.54	9.54	0.97

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Table 2. Bacterial Strain MTCC 5093 is unable to neutralize the beverage waste water when inoculated individually

Source	Original pH of Waste Water	After 2 days of incubation		After 4 days of incubation		After 7 days of incubation	
		pH	Change in pH unit	pH	Change in pH unit	pH	Change in pH unit
Without carbohydrates	12.43	12.42	0.01	12.41	0.02	12.41	0.02
	11.50	11.48	0.02	11.47	0..03	11.46	0.04
	10.91	10.83	0.05	10.82	0.07	10.83	0.08
	10.51	10.46	0.05	10.44	0.07	10.41	0.10
1% Sucrose	12.12	12.09	0.03	12.09	0.03	12.08	0.04
	11.50	11.47	0.03	11.43	0.07	11.41	0.09
	10.91	10.87	0.04	10.85	0.06	10.83	0.08
	10.51	10.47	0.04	10.42	0.09	10.39	0.12
1% Glucose	12.22	12.20	0.02	12.18	0.04	12.18	0.04
	11.50	11.42	0.08	11.40	0.10	11.38	0.12
	10.91	10.29	0.62	10.21	0.70	10.13	0.78
	10.51	10.05	0.46	9.82	0.69	9.50	1.01

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Table 3 Bacterial Strain MTCC 5092 and MTCC 5093 are unable to neutralize the beverage waste water even when inoculated t gether as a cons rtium

Source	Original pH of Waste Water	After 2 days of incubation		After 4 days of incubation		After 7 days of incubation	
		pH	Change in pH unit	pH	Change in pH unit	pH	Change in pH unit
Without carbohydrates	12.43	12.43	0.00	12.42	0.01	12.42	0.01
	11.50	11.48	0.02	11.46	0..04	11.40	0.08
	10.91	10.84	0.05	10.83	0.08	10.82	0..09
	10.51	10.43	0.08	10.37	0.14	10.37	0.14
1% Sucrose	12.12	12.11	0.01	12.11	0.01	12.10	0.02
	11.50	11.42	0.08	11.41	0.09	11.26	0.24
	10.91	10.71	0.20	10.57	0.34	10.03	0.88
	10.51	10.18	0.33	9.83	0.68	9.51	1.00
1% Glucose	12.22	12.20	0.02	12.16	0.06	12.16	0.06
	11.50	11.38	0.12	10.82	0.68	10.62	0.88
	10.91	9.93	0.98	9.91	1.00	9.69	1.22
	10.51	9.54	0.97	9.06	1.45	8. 84	1.67

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Thus, one can conclude that the function of consortia of the instant Application is selective in nature. A particular and non-obvious process prepares the consortium.

9. I tried to bring about acclimatization at pH higher than 11. Here, the aim was to achieve better results than the results of the instant Application. However, the same acclimatization process failed for the same strains at higher pH **(results shown,Table Y and Z)**. Thus, even the acclimatization-step, which is critical for the success of the instant process is selective in nature. So, it is not universal that any/all alkalophilic bacteria thrive in any complex media at any pH and also decrease the pH to desired levels.
10. The sequence of events including selection of particular strains, acclimatizing these particular strains at a particular pH of 11, and mixing the acclimatized strains of a particular pH to obtain a desired consortium is contributing in totality to produce desired results. Each of the aforementioned events is critical for the desired results. The above-shown data clearly establishes the same. Further, the sequence of events is also extremely critical. The acclimatization of the strains individually has its significance. Further, the subsequent mixing of the acclimatized strains is totally non-obvious.

11. It is not obvious for a person skilled in the art to predict the behaviour of the microbial strains including alkalophilic bacteria unless a skilled person actually conducts the experiments, the person cannot be sure about achieving the desired result. The inventors have conducted multiple experiments with strains of varying nature . It is only after years of hard work and intellectual capability that the inventor has been able to identify the strains of the instant application. The strains meet all the requirements of patentability including novelty and inventiveness.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements

TABLE Y

ACCLIMATIZATION STUDIES OF *BACILLUS ALKALOPHILUS* AT DIFFERENT pH (ABOVE 11.0) W.R.T. TIME IN TERMS OF OPTICAL DENSITY AT 650 NM AND VIABILITY

Time (days)	pH											
	11.0		11.2		11.4		11.6		11.8		12.0	
	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability
2	0.275	+	0.116	-	0.114	-	0.102	-	0.100	-	0.090	-
4	0.282	+	0.114	-	0.099	-	0.095	-	0.093	-	0.092	-
6	0.299	+	0.109	-	0.105	-	0.100	-	0.097	-	0.089	-
8	0.382	+	0.106	-	0.102	-	0.100	-	0.100	-	0.090	-
10	0.401	+	0.118	-	0.105	-	0.104	-	0.099	-	0.087	-
12	0.485	+	0.120	-	0.115	-	0.106	-	0.090	-	0.089	-
14	0.532	+	0.100	-	0.099	-	0.100	-	0.098	-	0.090	-

TABLE Z

ACCLIMATIZATION STUDIES OF *BACILLUS SP.* AT DIFFERENT pH (ABOVE 11.0) W.R.T. TIME IN TERMS OF OPTICAL DENSITY AT 650 NM AND VIABILITY

Time (days)	pH											
	11.0		11.2		11.4		11.6		11.8		12.0	
	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability	OD_{650}	viability
2	0.275	+	0.118	-	0.109	-	0.110	-	0.109	-	0.100	-
4	0.282	+	0.117	-	0.110	-	0.105	-	0.108	-	0.099	-
6	0.299	+	0.114	-	0.112	-	0.106	-	0.105	-	0.097	-
8	0.382	+	0.108	-	0.109	-	0.112	-	0.106	-	0.090	-
10	0.401	+	0.105	-	0.105	-	0.109	-	0.110	-	0.092	-
12	0.485	+	0.107	-	0.099	-	0.098	-	0.107	-	0.093	-
14	0.532	+	0.100	-	0.095	-	0.099	-	0.099	-	0.099	-

Acclimatization of both bacterial strains, i.e., *Bacillus alkalophilus* and *Bacillus sp.*, individually to different pH values, in modified Tryptose Soya Broth (TSB), was tried by sub-culturing the said strains a number of times at pH values in the increasing order above 11.0. All the higher pH sets were also run parallely, in order to see if there was any difference in results. The said bacterial strains, however, were able to grow well only at pH 11.0, as presented in tables Y and Z.

Results in tables Y and Z show that optical density of the cultures did not show any change upon exposure to higher pH, implying that there was no increase in cell numbers. These results were further authenticated by viabilty studies. Viability testing of the cultures subjected to acclimatization, showed that no growth was exhibited in the cultures and that they had been rendered non-viable by being exposed to higher pH.

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are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated: 31/X/2003

RITA KUMAR

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MTCC

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To
Dr. Rita Kumar
Institute of Genomics & Integrative Biology
(IGIB), Delhi University Campus
Mall Road, DELHI- 110 007

NAME AND ADDRESS OF THE DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR: Strain 1-Bacillus alkalophilus

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: MTCC 5092

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by :

(X) a scientific description

(X) a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above,

which was received by it on 03-03-2003 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION - NEW DEPOSIT

The microorganism identified under I above was received by this International Depositary Authority on _____ (date of the original deposit) and a request to convert the original deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Dr.Tapan Chakrabarti
Microbial Type Culture Collection & Gene Bank

Address: Institute of Microbial Technology
Sector 39-A, Chandigarh- 160 036
India

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 17.3.03

[1] Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired

MTCC

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To	
Dr. Rita Kumar Institute of Genomics & Integrative Biology (IGIB), Delhi University Campus Mall Road, DELHI- 110 007. NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED	VIABILITY STATEMENT issued pursuant to Rule 10.2 by the INTERNATIONAL DEPOSITARY AUTHORITY identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Dr. Rita Kumar Address: Institute of Genomics & Integrative Biology (IGIB), Delhi University Campus Mall Road, DELHI- 110 007.	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY MTCC 5092 Date of the deposit or of the transfer: 03-03-2003

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested

on [illegible] [2] on that date, the said microorganism was

(X) [3] viable

(___) [3] no longer viable

[1] Indicate the date of the original deposit or, where the new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box

[*] Form MTCC BP/9 (first page)

(16/24)

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[*]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Dr. Tapan Chakrabarti Microbial Type Culture & Gene Bank Address: Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036 India	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s): Date: 17.12.02

[*] Fill in if the information has been requested and if the results of the test were negative.

[†] Form MTCC BP/9 (second and last page)

17/24

MTCC

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To
Dr. Rita Kumar
Institute of Genomics & Integrative Biology
(IGIB), Delhi University Campus
Mall Road, DELHI- 110 007

NAME AND ADDRESS OF THE DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:
Strain 2- Bacillus species

Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
MTCC 5093

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by :

(X) a scientific description

(X) a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above,

which was received by it on 03-03-2003 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION - NEW DEPOSIT

The microorganism identified under I above was received by this International Depositary Authority on _____ (date of the original deposit) and a request to convert the original deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Dr.Tapan Chakrabarti

Microbial Type Culture Collection & Gene Bank

Address: Institute of Microbial Technology
Sector 39-A, Chandigarh- 160 036
India

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Tapan Chakrabarti

Date: 17/2/03

[1] Where Rule 6.4(d) applies, such date is the date on which the status of International Depositary Authority was acquired

[2] Form MTCC BP/4 (sole page)

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MTCC

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To	
Dr. Rita Kumar Institute of Genomics & Integrative Biology (IGIB), Delhi University Campus Mall Road, DELHI- 110 007. NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED	VIABILITY STATEMENT issued pursuant to Rule 10.2 by the INTERNATIONAL DEPOSITARY AUTHORITY Identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Dr. Rita Kumar Address: Institute of Genomics & Integrative Biology (IGIB), Delhi University Campus Mall Road, DELHI- 110 007.	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY MTCC 5093 Date of the deposit or of the transfer: 03-03-2003

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested

on 12-03-2003 [2] on that date, the said microorganism was

(X) [3] viable

( ) [3] no longer viable

[1] Indicate the date of the original deposit or, where the new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box

[1] Form MTCC BP/9 (first page)

19/24

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Dr. Tapan Chakrabarti Microbial Type Culture & Gene Bank Address: Institute of Microbial Technology Sector 39-A, Chandigarh - 160 036 India	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s): Date: 17.12.03

[4] Fill in if the information has been requested and if the results of the test were negative.

[1] Form MTCC BP/9 (second and last page)

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International Journal of Systematic Bacteriology, Vol 46, 759-764, Copyright © 1996 by the International Union of Microbiological Societies

ARTICLES

Bacillus sporothermodurans, a new species producing highly heat- resistant endospores

B Pettersson, F Lembke, P Hammer, E Stackebrandt and FG Priest
Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

Bacteria that differentiate into highly heat-resistant endospores (HHRS strains) may survive ultrahigh-temperature treatment of milk and germinate in the final product. They do not noticeably spoil the milk and are nonpathogenic. The complete (>96%) 16S rRNA genes from three HHRS strains were identical, and phylogenetic analysis placed them alongside Bacillus firmus in the B. megaterium group of the genus Bacillus. Moreover, the approximately 550 nucleotides between regions U2 and U5 were invariant for seven HHRS strains. However, three cloned 16S rRNA genes from one HHRS strain, M215, showed marked size and sequence variations within the V1 and V2 regions. DNA reassociation assays confirmed the distinction between a reference HHRS strain and closely related members of the B. megaterium group, notably, B. firmus (30%), B. benzoevorans (28%), and B. circulans (20%). Ribotyping and pyrolysis mass spectrometry both indicated that the HHRS strains belong to a homogeneous, species-ranked taxon, an exception being strain TP1248, which is slightly atypical. The HHRS strains are unusual in that they grow poorly, if at all, on nutrient agar; good growth is obtained on brain heart infusion agar. On subculture, most HHRS strains form long, filamentous rods which stain unevenly in the Gram reaction. [redacted] We propose the name Bacillus sporothermodurans for these bacteria, which are phenotypically and phylogenetically distinct from other Bacillus species. The type strain is M215 (= DSMZ 10599).

CLASSIFICATION

M. luteus is most closely related to M. lylae, from which it can be differentiated by the latter not growing on inorganic nitrogen agar, many M. lylae strains being resistant to lysozyme and differences in peptidoglycan type and amino sugar composition within the structure of the cell wall. The GC content of the DNA is 65-75 mol%.

BIOCHEMISTRY

M. luteus produces yellow to cream-white water insoluble pigments. It is a strict aerobe. Most strains can grow on inorganic nitrogen agar and few can reduce nitrate. They are oxidase positive. M. luteus will not produce acid from glucose or glycerol under aerobic conditions and not produce arginine dihydrolase or β-galactosidase. Carbohydrates are oxidized to CO_2 and water.

GENETICS

There is no close genetic relationship between the species on the basis of DNA hybridization studies. M. luteus differs from M. lylae by 40-50% and from the other species a similarity of only 10-18% was noted.

It was shown that genetic exchange could occur in this genus in the 1960's. These studies led to the development of optimal conditions to effect this "tranformation". On the basis of these transformation studies parts of the M. luteus genome have been mapped. The genes for the biosynthetic pathways for tryptophan and histidine have been mapped.

Up to about half the strains have been shown to carry plasmids

varying in size from 1 to 100MDa.

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E. corrodens: Since only 19 laboratories would have performed susceptibility testing on this isolate the results were not graded. Most of the 15 laboratories that indicated testing by Kirby Bauer noted that there are currently no standards for interpreting these results. Four laboratories used Etest. Nine laboratories indicated they would include a comment on the rep rt recommending penicillin therapy or alternatively ampicillin, imipenem, trimethoprim- sulfamethoxazole (SXT), or erythromycin. Also, they would state that Eikenella are usually resistant to clindamycin, oxacillin, first generation cephalosporins and aminoglycosides. The remaining laboratories either did not address antibiotic susceptibility testing or indicated that they would refer the isolate for testing.

Most Eikenella strains are susceptible to ampicillin, ticarcillin, imipenem/meropenem, tetracycline and chloramphenicol and are resistant to oxacillin, clindamycin, vancomycin, erythromycin, metronidazole and the aminoglycosides. Most isolates are variably sensitive to first generation cephalosporins. Empiric oral therapy in the non-penicillin allergic patient should be with ampicillin or ampicillin/clavulanate (Clavulin). Ceftriaxone is usually given for severe infections requiring parenteral therapy.

IDENTIFICATION Organisms of the B. fragilis grp. are nonmotile, anaerobic, gram-negative rods with rounded ends. Colonies on anaerobic blood agar are 1-4 mm in diameter, nonhemolytic, gray, and semi-opaque with concentric whorls inside the colonies. A key characteristic of this group is that growth is enhanced by bile. They are all resistant to penicillin and kanamycin, but sensitive to rifampin by the disc technique. All are saccharolytic, and their carbohydrate fermentation patterns along with indole help to differentiate between the species within this group.

Eikenella is a fastidious gram-negative bacterium that is part of the HACEK group of organisms. E. corrodens can be easily recovered on blood and chocolate agar but it does not grow on MacConkey agar. X and V factors are not required for growth although hemin is necessary for aerobic growth. Colonies are small after 48 hours and 50% may cause pitting of the agar. Corroding and non-corroding strains can be found on the same plate. Most strains produce an odor suggestive of "bleach" and a pale yellow pigment is usually produced. A Gram stained slide of the colony shows slender gram-negative bacilli or coccobacilli with rounded ends. E. corrodens typically is oxidase-positive, catalase-negative (rare strains may be weakly catalase-positive), reduces nitrate to nitrite and does not produce acid from carbohydrates. It is ornithine decarboxylase-positive, which will help differentiate it from Capnocytophaga, Kingella and CDC group EF-4.

IJSEM Papers in Press – Published online 1 August 2003 as http://dx.doi.org/10.1099/ijs.0.02754-0

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Transfer of *Ralstonia eutropha* and related species t *Wautersia* gen. nov.: proposal of *Wautersia basilensis* Steinle *et al.* 1999, comb. nov., *W. eutropha* Yabuuchi *et al.* 1996, comb. nov., *W. gilardii* Coenye *et al.* 1999, comb. nov., *W. metallidurans* Goris *et al.* 2001, comb.nov., *W. oxalatica* Sahin *et al.* 2000, comb. nov., *W. paucula* Vandamme *et al.*, comb. nov., *W. respiraculi* Coenye *et al.* 2003, comb. nov. and *W. taiwanensis* Chen *et al.* 2001, comb. nov., emended description of *Ralstonia insidiosa* Coenye *et al.* 2003 and proposal of *Ralstonia [Pseudomonas] syzygii* Roberts *et al.*, 1990, comb. nov.

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Keywords: *Ralstonia*, *Wautersia*

Running title: *Wautersia* gen. nov.

Genbank accession numbers: The GenBank accession numbers for the 16S rRNA gene sequences of the *R. insidiosa* strains CCUG 46389 and CCUG 46388 are AJ507103 and AJ539233.

The results of lactose and maltose acidification by *R. pickettii* strains, the 16S rRNA gene signature sequences of the *Ralstonia* species, detailed phenotypic data of different species and a similarity matrix of the 16S rDNA sequences are provided as supplementary material (respectively Tables A1, A2, A3, A4) in the IJSEM Online version of this paper at http://ijs.sgmjournals.org.).

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ABSTRACT

Comparative 16S rDNA sequence analysis indicates that two distinct sublineages, with a sequence dissimilarity of more than 4% (bootstrap value 100%), exist within the genus *Ralstonia:* the *R. eutropha* lineage comprising *R. basilensis, R. campinensis, R. eutropha, R. gilardii, R. metallidurans, R. oxalatica, R. paucula, R. respiraculi* and *R. taiwanensis* and the *R. pickettii* lineage, comprising *R. insidiosa, R. mannitolilytica, R. pickettii, R. solanacearum* and *R. syzygii* comb. nov. This phylogenetic discrimination is supported by phenotypic differences: members of the *R. eutropha* lineage have peritrichous flagella, do not produce acids from carbohydrates and are colistin sensitive, in contrast to members of the *R. pickettii* lineage which have one or more polar flagella, produce acid from several carbohydrates and are colistin resistant. Members of the *R. pickettii* lineage are viable up to six days on Tryptic Soy Agar at 25°C, while members of the *R. eutropha* lineage are viable for more than nine days. It is proposed to reclassify the *R. eutropha* lineage species into a new genus, for which the name *Wautersia* gen. nov. is proposed. Finally, based on literature and on new DNA-DNA hybridisation data, it is proposed to rename *Pseudomonas syzygii* as *Ralstonia syzygii,* comb. nov.